

Claims:

1. A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature,  $T_1$ , for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature,  $T_2$ , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature,  $T_3$ , provided that when priming and primer extension product formation are carried out in the same step,  $T_2$  and  $T_3$  are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic polymeric volume exclusion agent.

2. A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature,  $T_1$ , for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature,  $T_2$ , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension

products in a reaction mixture of PCR reagents, by incubation at a third temperature,  $T_3$ , provided that when priming and primer extension product formation are carried out in the same step,  $T_2$  and  $T_3$  are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a DNA polymerase and a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

3. A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature,  $T_1$ , for denaturation of the strands of the target nucleic acids or their primer extension products,

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature,  $T_2$ , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature,  $T_3$ , provided that when priming and primer extension product formation are carried out in the same step,  $T_2$  and  $T_3$  are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a hot start DNA polymerase and a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridisation.

4. A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature,  $T_1$ , for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature,  $T_2$ , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature,  $T_3$ , provided that when priming and primer extension product formation are carried out in the same step,  $T_2$  and  $T_3$  are the same, and

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a hot start DNA polymerase and optionally a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridisation, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

5. A method according to one of claims 1 – 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate.

6. A method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula:



wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000.

7. The method according to claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylene, 1,3-butylene, or 1,2-hexylene.

8. The method according to claim 6, characterized in that the polyether is poly(ethylene glycol).

9. The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 1000 to 2,000,000.

10. The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 to 500,000.

11. The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000.

12. The method according to claim 5, characterized in that the volume exclusion reagent is a dextran.

13. The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000.

14. The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000.

15. The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 to 60,000.

16. The method according to claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate).

17. An amplification reaction composition which is buffered to a pH of from about 7.5 to about 9, comprising:

one or more sets of primers,

a thermostable hot-start-DNA polymerase,

a plurality of dNTP's, and

1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, and optionally a probe.

18. The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 15 weight % of a nonionic, polymeric volume exclusion agent.

19. The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent.

20. A kit for the coamplification of two or more target nucleic acids comprising:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,

a thermostable hot-start DNA polymerase,

a plurality of dNTP's, and

1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and

(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate.

21. A self-contained test device comprising, in separate compartments:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,

a thermostable hot-start DNA polymerase,  
a plurality of dNTP's, and  
1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a  
nonionic, polymeric volume exclusion agent, and,  
(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble  
substrate, the compartments being connected in the test device so that the amplification  
reaction composition can be brought into contact with the capture reagent after amplification  
without opening the test device.

22. A kit for preparing an amplification reaction composition according to claim 17  
comprising:  
at least one hot-start DNA polymerase, and  
at least one polymeric exclusion reagent.